reaction proceeded at 0°C for 20min and was terminated by addition of 0.4% phenol (5ml), which rapidly exhausts any remaining diazonium groups. CaCl₂ appears to be necessary to preserve enzymic activity.

The insoluble product is almost identical in pH and reaction temperature optima with the soluble enzyme, but is much more stable. It has retained complete activity during storage for several months at 4°C, compared with a decline of about 25%/day for the soluble enzyme stored under identical conditions.

Apparent recovery of enzymic activity depends on the size of substrate used to measure proteolysis. High-molecular-weight substrates appear to have sterically restricted access to the catalytic sites. Such restriction is progressively decreased as the molecular weight (or shape and size) of a substrate falls. With the exception of one substrate, all activity was measured by determination of trichloroacetic acid-soluble peptide released after 30min at 37°C. The exception was that of L-leucine *p*-nitroanilide [Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.], where release of *p*-nitroaniline was followed at 400nm.

A simple expression (1) seems to relate the molecular weight of a substrate to the soluble enzyme/insoluble enzyme reaction-rate ratio, and applies over a wide size range from proteins to simple peptides:

$$\frac{\text{Reaction rate with insoluble enzyme}}{\text{Reaction rate with soluble enzyme}} = \frac{K + k \cdot \log(\text{mol.wt. of substrate})}{1} \quad (1)$$

With low-molecular-weight substrates approx. 75% of catalytic activity is retained.

The use of insoluble catalyst has two principal advantages over the soluble protein. First, the stability referred to above obviates the necessity for repeated addition of enzyme to combat self-destruction. Secondly, its simple removal from reaction mixtures by centrifugation overcomes the problem of contamination of digests by enzyme-derived peptides.

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Glycoproteins from Yeast Cell Wall

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Yeast cells, of various strains and at various stages of growth, were suspended in water and treated for

2min in a Braun disintegrator, and cell walls were isolated by differential centrifugation. Fractionation with ethylenediamine gave the fractions A, B and C described by Korn & Northcote (1960). Chemical analyses of the fractions from the different strains showed little variation, although walls with a high affinity for calcium had a fraction A with high phosphorus content. Polyacrylamide-gel electrophoresis at pH 8.3 in miniature columns (Davis, 1964) separated fraction A into two sharp bands. Both bands took up stains for protein (Amido Black) and carbohydrate (Schiff-periodate), and the relative proportions of the bands depended on the yeast strain and the stage of growth. From yeast cell walls labelled with [³²P]phosphate it was shown that no phosphorus was present in one of the bands. Preparative polyacrylamide-gel electrophoresis was used to isolate quantities of both band components. For fraction B, a complex insoluble in water, gel electrophoresis at pH 8.6 in 10% Cyanogum and 8M-urea gave two components staining for protein and carbohydrate.

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ε-N-Monomethyl-lysine and Trimethyl-lysine in Myosin

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It has been reported by Hardy & Perry (1969) that subfragment I of myosin and the total protein fraction of myofibrils contain ε-N-monomethyl-lysine. In addition to the latter and 3-methylhistidine, Hardy & Perry (1969) reported the separation of a fraction containing an unidentified methylated amino acid. This has now been identified as ε-N-trimethyl-lysine (DeLange, Glazer & Smith, 1969) by comparison of its chromatographic behaviour in the Unicrome Beckman amino acid analyser (0.35M-sodium citrate buffer, pH 5.79, 28°C) with a known sample of the methylated amino acid (kindly supplied by Dr R. J. DeLange). No dimethyl-lysine could be detected in subfragment I. The presence of ε-N-monomethyl-lysine in subfragment I received confirmation from the independent observations by Huszar & Elzinga (1969), who obtained a single peak of elution characteristics identical with those of ε-N-monomethyl-lysine, although their analytical procedure did not resolve the N-methyl-lysines.

Myosin and subfragment I contain 0.24mol and 0.59mol of ε-N-monomethyl-lysine respectively and 0.72mol and 1.75mol of ε-N-trimethyl-lysine respectively/10⁵g. Whereas 3-methylhistidine, the other methylated amino acid present in adult skeletal-muscle myosin, is absent from myosin isolated from